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Development of Improved Crosslinking Monomers for Molecularly Imprinted Materials.

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ABSTRACT

Molecular imprinting involves the self-assembled complexation of a substrate to functional monomers to form a pre-polymer complex which is "locked-in" to place by copolymerization with an excess of crosslinking monomer. Removal of the template leaves binding or catalytic sites that are complementary in size, shape, and functionality to the template. Most of the research in molecularly imprinted materials has focused on choice of substrate or functional monomer of the pre-polymer complex. The cross-linking monomers have primarily been EGDMA or DVB, which are commercially available. Redirecting focus on the design of crosslinking monomers for molecular imprinting, we have developed new classes of crosslinked polymers to optimize the performance of molecularly imprinted polymers. The design of the new crosslinking monomers has followed two strategies: (1) development of new crosslinked materials for formation of the supporting matrix, and (2) development of crosslinking monomers that simultaneously serve as the functional monomer. The details of the design, synthesis, polymerization and performance of these new crosslinking monomers for molecularly imprinted polymers will be reported.

INTRODUCTION

Currently, cross-linking monomers for molecularly imprinted polymers (MIPs) have primarily been EGDMA or DVB, which are commercially available.[1-2] One benefit of using these monomers is that they are inexpensive and readily available in large quantities. This is important for applications that would require large amounts of material such as industrial catalytic reactors or separations on the industrial scale. However, many future applications of imprinted polymers are envisioned in the fields of microfabricated sensors and microseparations that will only require small amounts of material. Therefore, economic price considerations of the imprinting materials is less of a concern. Instead, materials with the best performance possible are the target for microfabricated and nanofabricated devices.

Most of the research in molecularly imprinted materials has focused on choice of substrate or functional monomer of the pre-polymer complex. However, approximately 80-90% of the imprinted polymers are composed of the crosslinking monomer, with the remaining 10-20% comprised of functional monomer. The large percentage of crosslinking monomer materials in imprinted polymers affords the possibility of a commensurate improvement in polymer properties. Redirecting focus on the design of crosslinking monomers for molecular imprinting, we have developed new classes of crosslinked polymers to optimize the performance of molecularly imprinted polymers.

EXPERIMENTAL DETAILS

Preparation of Dansyl-L-phenylalanine imprinted polymer using novel crosslinking monomer N,O-bismethacryloyl ethanolamine (NOBE). The following procedure was used for imprinted polymers employing the new cross-linking monomers. In a 13 x 100 mm test tube, (0.064g, 0.16 mmol) of dansyl-L-phenylalanine was dissolved in 1.5 mL of ACN. To this solution was added (1.3g, 6.6 mmol) of **NOBE**, (0.113g, 1.32 mmol) of MAA, and (0.021g, 0.13 mmol) of AIBN. For comparison to traditionally formulated imprinted polymers, another polymer was imprinted using the formulation above, substituting EGDMA as the crosslinking monomer. The solution was purged by bubbling nitrogen gas into the mixture for 5 minutes, then capped and sealed with teflon tape and parafilm. The samples were inserted into a photochemical turntable reactor (ACE Glass Inc.) which was immersed in a constant temperature bath. A standard laboratory UV light source (a Canrad-Hanovia medium pressure 450 W mercury arc lamp) jacketed in a borosilicate double-walled immersion well was placed at the center of the turntable. The polymerization was initiated photochemically at 20°C and the temperature maintained by both the cooling jacket surrounding the lamp and the constant temperature bath holding the entire apparatus. The polymerization was allowed to proceed for 10 h, then used for chromatographic experiments.

Preparation of S-(-)-nicotine imprinted polymer using functionalized crosslinking monomer N,O-bismethacryloyl aspartic acid (NOAA). In a borosilicate scintillation vial, (0.0454g, 0.28 mmol) of S-(+)-Nicotine was dissolved in 0.95 mL methylene chloride. To this solution was added (0.991g, 5.0 mmol) EGDMA, (0.135g, 0.53 mmol) of functional monomer **NOAA**, and (0.105g, 0.64 mmol) AIBN. The control polymer was formulated in a similar fashion, without introduction of a template molecule. For comparison to traditionally formulated imprinted polymers, another polymer was imprinted using the formulation above, only substituting (0.045g, 0.53 mmol) methacrylic acid (MAA) in place of the aspartic acid functional monomer. The solution was purged by bubbling nitrogen gas into the mixture for 5 minutes, then capped and sealed with teflon tape and parafilm. The samples were inserted into a photochemical turntable reactor (ACE Glass Inc.) which was immersed in a constant temperature bath. A standard laboratory UV light source (a Canrad-Hanovia medium pressure 450 W mercury arc lamp) jacketed in a borosilicate double-walled immersion well was placed at the center of the turntable. The polymerization was initiated photochemically at 20°C and the temperature maintained by both the cooling jacket surrounding the lamp and the constant temperature bath holding the entire apparatus. The polymerization was allowed to proceed for 10 h, then used for chromatographic experiments.

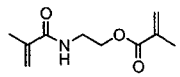
Chromatographic Experiments. The polymers were ground using a mortar and pestle, the particles were sized using U.S.A. Standard Testing Sieves (VWR), and the fraction between 20-25 μm was collected. The particles were slurry packed, using a Beckman 1108 Solvent Delivery Module, into stainless steel columns (length, 7.5 cm, i.d. 2.1 mm) to full volume for chromatographic experiments. The polymers were then washed on line for 12 hours using acetonitrile/acetic acid: 90/10, at a flow rate of 0.1 mL/min to remove the template. HPLC analyses were performed isocratically at room temperature (21°C) using a Hitachi L-7100 pump

with a Hitachi L-7400 detector. The flow rate, UV detector wavelength, substrate and substrate concentration are provided with the tables in the text. The void volume was determined using acetone as an inert substrate. The separation factors (α) were measured as the ratio of capacity factors (k'_S/k'_R). The capacity factors were determined by the relation $k' = (R_v - D_v) / D_v$, where R_v is the retention volume of the substrate, and D_v is the void volume.

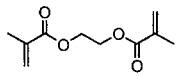
DISCUSSION

Two types of novel crosslinking monomers are currently being investigated by our lab. First, crosslinking monomers are used for copolymerization of the pre-polymer complex forming the the required highly-crosslinked network polymer. In this case, the crosslinker provides the matrix material that holds the functional monomers provided in place. Wulff and coworkers have shown that maximization of crosslinker improves the quality of imprinted polymers. This often leads to finding a balance between crosslinker and non-crosslinking functional monomers, in order to optimize the performance of imprinted polymers. Too much of one or the other can lead to undesirable results. Therefore, it is postulated that properties of molecularly imprinted materials could be enhanced by combining the crosslinking and functional properties needed into one monomer. This is the second type of monomer format currently being investigated by our laboratory.

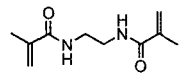
An example of the first type of monomer is N,O-bismethacryloyl ethanolamine (**NOBE**), shown in figure 1. The structure of this monomer is similar to the commonly used ethyleneglycol dimethacrylate (**EGDMA**); however, it incorporates an acrylamide group instead of a methacrylate group that may provide different binding interactions with template molecules, and different polymer morphology characteristics.



NOBE



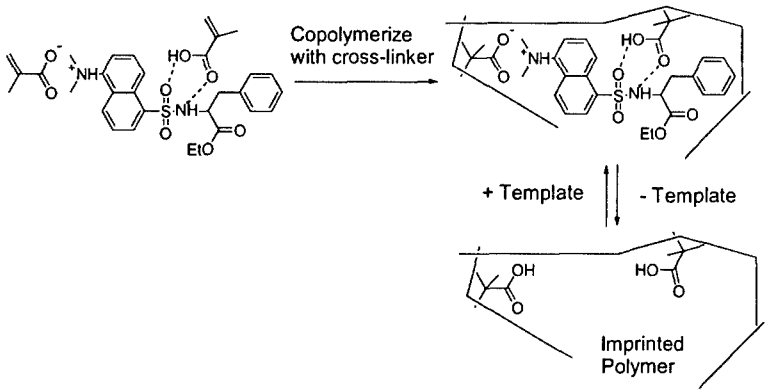
EGDMA



EDBMP

Figure 1. Example of crosslinking monomers: **NOBE** = novel crosslinking monomer N,O-bismethacryloyl ethanolamine; **EGDMA** = ethylene glycol dimethacrylate; **EDBMP** = N,N'-1,2 ethanediylbis(2-methyl-2-propenamide).

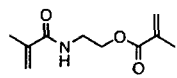
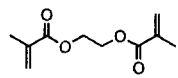
Furthermore, **NOBE** is soluble in non-polar organic solvents such as acetonitrile and chloroform or methylene chloride, versus the similar **EDBMP** reported in the literature to be insoluble in these solvents.[3] To test the performance of **NOBE**, polymers were imprinted with Dansyl-L-phenylalanine as illustrated in scheme 1, with chloroform as the solvent. An HPLC column



Scheme 1. Outline of strategy for imprinting Dansyl-L-phenylalanine.

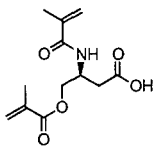
made with this polymer was compared to a column made with **EGDMA** under the same conditions, and the results are shown in table 1. The new crosslinking monomer provided polymers with increased binding affinity versus polymers formed with **EGDMA**, verified by the large increase in capacity factors. More important, increased chiral selectivity was seen under these conditions for the L enantiomer of Dansylphenylalanine, versus the D enantiomer.

Table 1. Binding data for Dansyl-L-phenylalanine imprinted polymers.*

Entry	Crosslinking Monomer	k'_L	k'_D	α
1	 **NOBE**	1.5	0.8	1.9
2	 **EGDMA**	0.1	0.1	1.0

* Mobile phase = 99/1: MeCN/HOAc, flow rate = 1.0 mL/min, substrate samples consisted of 5 μL injections of a 0.1 mM Dansyl-L-phe substrate concentration, detection at λ = 330 nm.

An example of the second type of monomer is N,O-bismethacryloyl aspartic acid (**NOAA**) shown in figure 2. Inspired by proteins in nature that are responsible for molecular recognition and catalysis, functional crosslinking monomer **NOAA** was designed based on the amino acid aspartic acid. Polymers were synthesized using both the **NOAA** functional monomer and with the traditionally employed methacrylic acid (**MAA**) for comparison. Polymers were imprinted

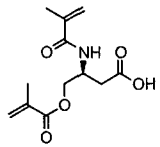


NOAA

Figure 2. Example of a functionalized crosslinking monomer, N,O-bismethacryloyl aspartic acid (**NOAA**).

with the naturally occuring S enantiomer of nicotine as the template, and the results for binding of both enantiomers of nicotine are shown in table 2. Looking at the table, enantioselectivity is enhanced by almost an order of magnitude for polymer made with functional monomer **NOAA** versus traditional methacrylic acid (**MAA**) functionalized polymers. The increase is postulated to arise primarily from using a crosslinking carboxylic acid functional monomer versus the non-crosslinking methacrylic acid, with minor enhancement to binding from the amide moiety as precedented by the **NOBE** data above.

Table 2. Binding data for S-(-)-nicotine imprinted polymers.*

Entry	Crosslinking Monomer	k'_S	k'_R	α
1	 **NOAA**	4.0	0.2	20.0
2	 **MAA**	0.7	0.2	3.5

* Mobile phase = 99/1: MeCN/HOAc, flow rate = 0.1 mL/min, substrate samples consisted of 10 μL injections of a 1.0 S-(-)-nicotine substrate concentration, detection at λ = 262 nm.

In addition to increased crosslinking, the polymers made with the new monomers more closely resemble protein matrices that perform molecular recognition functions seen in enzymes and antibodies, which may account for increased binding characteristics. The increased performance of these new materials is anticipated to improve the role of imprinted polymers for sensors, catalysts and separation science.

REFERENCES

1. B. Sellergren, Ed., *Molecularly Imprinted Polymers. Man-Made Mimics of Antibodies and their Application in Analytical Chemistry* (Elsevier Science, Amsterdam, The Netherlands, 2000).

2. G. Wulff, Angew. Chem. Int. Ed. **34**, 1812 (1995).

3. Shea, K.J., Stoddard, G.J., Shavelle, D.M., Wakui, F., Choate, R.M., Macromolecules, **23**, 4497 (1990).